PATENT ABSTRACTS OF JAPAN

(11)Publication number : **2000-063400**
(43)Date of publication of application : **29.02.2000**

(51)Int.Cl. **C07K 14/435**
A01N 63/00
A23L 3/3526
// C12N 15/09

(21)Application number : **10-239435**
(22)Date of filing : **12.08.1998**

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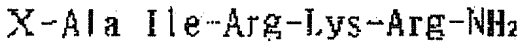
(54) ANTIMICROBIAL PEPTIDE AND ANTIMICROBIAL AGENT COMPRISING THE SAME AS ACTIVE INGREDIENT

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain a new antimicrobial peptide having a low molecular weight, a wide antimicrobial spectrum, excellent safety and antimicrobial activity and slight influence of action of immune system in the body.
SOLUTION: This peptide is shown by the formula (X is a peptide obtained by peptide bond between one amino acid residue or two or more amino acid residues and is selected from the group consisting of Ala-Leu-Arg-Leu, Ala-Leu- Leu-Leu, Ala-Trp-Leu-Leu, Ala-Leu-Tyr-Leu and Ala-Leu-Trp-Leu; Ag-NH2 shows the carboxyl group of Arg is amidated) such as Ala-leuu-Arg-Lys-Arg- NH2. The antimicrobial peptide of the formula is obtained by successively reacting fixed amino acids by a solid phase method.



LEGAL STATUS

[Date of request for examination]	30.08.1999
[Date of sending the examiner's decision of rejection]	
[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]	
[Date of final disposal for application]	
[Patent number]	3273314
[Date of registration]	01.02.2002
[Number of appeal against examiner's decision	

of rejection]
[Date of requesting appeal against examiner's decision of rejection]
[Date of extinction of right]

* NOTICES *

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CLAIMS

[Claim(s)]
[Claim 1] The following type (I)
X-Ala-Ile-Arg-Lys-Arg-NH2 (I)
It is the antibacterial peptide expressed with (X is a peptide one amino acid residue or two or more amino acid residue come to carry out peptide linkage among a formula, and it is shown that this carboxyl group of Arg has amidated Arg-NH2).
[Claim 2] The antibacterial peptide according to claim 1 whose number of the amino acid residue in X is three pieces or four pieces.
[Claim 3] The antibacterial peptide according to claim 1 whose number of the amino acid residue in X is four pieces.
[Claim 4] The antibacterial peptide according to claim 3 whose X is Ala-Xaa1-Xaa2-Leu (Xaa1 and Xaa (s)2 are amino acid residue other than the acidic amino acid which may be the same or may differ).
[Claim 5] X -- Ala-Leu-Arg-Leu, Ala-Leu-Leu-Leu, Ala-Trp-Leu-Leu, and Ala-Leu-Tyr-Leu And Ala-Leu-Trp-Leu from -- antibacterial peptide according to claim 3 which is a peptide of one of amino acid sequences chosen from the becoming group.
[Claim 6] The antimicrobial agent which makes an active principle claim 1 thru/or an antibacterial peptide given [of 5 / one] in a claim.
[Claim 7] The antimicrobial agent according to claim 6 whose antimicrobial agent is an edible antimicrobial agent.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention is invention of the technical field about the antimicrobial agent which makes an antibacterial peptide thru/or an antibacterial substance an active principle.
[0002]
[Description of the Prior Art] A citizen's clean / comfortable intention of "wanting to lead a clean and comfortable life" is further expanded under the effect of the latest E. coli bacillus O-157 etc. On the other hand, drug resistance bacteria, such as MRSA, are generated by extensive use of an antibiotic etc., and it is apprehensive about the future trend.
[0003] By current, including the antibiotic, various antimicrobial agents are offered and it is used not only in medical application but in various "antibacterial goods." As one of the flow of these antimicrobial agent development, its attention is paid to "an antibacterial peptide" and it is coming. This antibacterial peptide is a peptide which exists in sperm, such as mammalian, or a blood serum and has a broad antimicrobial spectrum, and is one of current [from the point of safety being high and it being assumed that a drug resistance bacterium cannot appear easily due to that use], and the antibacterial components to which its attention is paid most.
[0004] And existence of the antibacterial peptide originating in the body fluid of an insect is known as a kind of such an antibacterial peptide. That is, if bacteria and a corpuscle of a different kind are inoculated into an insect or a stimulus of giving a blemish for the body surface of an insect is given to it, it is known that various antibacterial peptides will be guided into the body fluid of these insects.
[0005] For example, the peptide (J. Biol.Chem.266 volume, 2455 - 24525 pages, 1991) with antimicrobial activity etc. is identified as biologically active peptide guided into kind (Zophobas atratus) larva body fluid of Tenebrionidae. Moreover, the physicochemical property is clarified also for the antibacterial peptide named the KOREOPUTE lysine (Coleoptericin). Furthermore, it is obtained from a kind of (Hyalophora cecropia) larva body fluid of Lepidoptera, and the antibacterial peptide belonging to a SEKURO pin group is known with the physicochemical property. It is thought that the antibacterial peptide in these insects has relation important for the biophylaxis of an insect without antibody sexuparaous ability also from having a broad antimicrobial spectrum.
[0006]
[Problem(s) to be Solved by the Invention] "Immunogenicity" is mentioned as one of the troubles about the above-mentioned antibacterial peptide. That is, since the above-mentioned antibacterial peptide will be captured by the immune system in the living body when it is prescribed for the patient into blood by making this into an antimicrobial agent, since the molecular weight is small as for neither, its inclination for the antibacterial action to be lost at an early stage is strong.
[0007] Moreover, it cannot be denied that there is a bias with it, but it is hard to say that the effective, antibacterial peptide large enough is offered to the both sides of gram positive bacterium and Gram negative bacterium. [a comparatively large but antimicrobial spectrum and] [a certain amount of] Furthermore, if this is applied to mammalian about the antibacterial peptide in an insect, possibility of

being accompanied by a certain cytotoxicity cannot be denied at all.
[0008] Then, the technical problem which this invention should solve is to offer the antibacterial means using an antibacterial peptide which conquered the above-mentioned trouble.
[0009]
[Means for Solving the Problem] this invention person repeated examination wholeheartedly as an antibacterial peptide of the above-mentioned insect origin paying attention to the structure and the function of an antibacterial peptide in the insect belonging to beetles, such as Scarabaeidae and a department of dust pellet SHIMUSHI, especially the Taiwan beetle (Oryctesrhinoceros) which is a kind of coca leaf BUTOMUSHI of Scarabaeidae, in order to solve this technical problem.
[0010] That is, the base sequence of the gene (henceforth a natural mold antibacterial peptide gene) which carries out the code of the antibacterial peptide in the larva of the Taiwan beetle was determined by giving a stimulus of a trauma etc. from the gene of the larva of the Taiwan beetle by which producing an antibacterial peptide in body fluid is known. From the base sequence of the antibacterial peptide gene determined here, the amino acid sequence of the antibacterial peptide (henceforth [unless it refuses especially] a natural mold antibacterial peptide) originating in the Taiwan beetle was presumed, and the invention of a new antibacterial peptide was aimed at based on the information about this amino acid sequence.
[0011] (1) The decision of the base sequence of a natural mold antibacterial peptide gene was usually made according to the method of determining the base sequence of a well-known gene about the decision of the base sequence of a natural mold antibacterial peptide gene.
[0012] That is, a natural mold antibacterial peptide is extracted and refined from the larva of ** Taiwan beetle. Determine the amino acid sequence of the amino terminal of this natural mold antibacterial peptide, and it carries out based on the amino acid sequence of the C terminal of the antibacterial peptide which further already originates in other insects other than the Taiwan beetle with that well-known amino acid sequence. the nucleotide chain which can carry out the code of the amino acid sequence of these amino terminals and C terminals -- preparing -- ** -- these nucleotide chains as a primer for gene amplification Gene amplification actuation (PCR method) of the part considered to carry out the code of the antibacterial peptide in the gene of the Taiwan beetle was performed, and the base sequence in the magnification product of the gene ** Obtained was determined.
[0013] In actuation of the above-mentioned **, separation and purification of the natural mold antibacterial peptide from the larva of the Taiwan beetle which determines the amino terminal of a natural mold antibacterial peptide and which is performed for accumulating were performed by [as being the following].
[0014] That is, after putting 3 ****** of the Taiwan beetle of ten animals on Hikami for several minutes and blunting the motion, the hypodermic needle was made to penetrate and a trauma was done. Subsequently, it bred for 24 to 48 hours in the container which kept the larva of the Taiwan beetle which did this trauma at 25 degrees C containing leaf mold. Then, the leg of a larva was cut and the body fluid drop of the larva of the Taiwan beetle which did a trauma into the tube of Hikami was extracted by pressing down and extracting an abdomen and performing ****.
[0015] Consequently, the body fluid of per larva of the Taiwan beetle of one animal and about 1.5 mL(s) was obtained. Thus, the obtained body fluid was given to centrifugal separation (39000xg) for 50 minutes, and the obtained supernatant liquid was saved at -20 degrees C except for the corpuscle component.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Drawing 1 is drawing showing the antimicrobial activity operation over the last purification profile and Staphylococcus aureus by the reverse phase column chromatography (SMART system) of a natural mold antibacterial peptide.
[Drawing 2] Drawing 2 is drawing by MALDI-TOF-MS showing the mass analysis of a natural mold antibacterial peptide.
[Drawing 3] Drawing 3 is drawing showing the concentration of the natural mold antibacterial peptide exerted on Staphylococcus-aureus growth.

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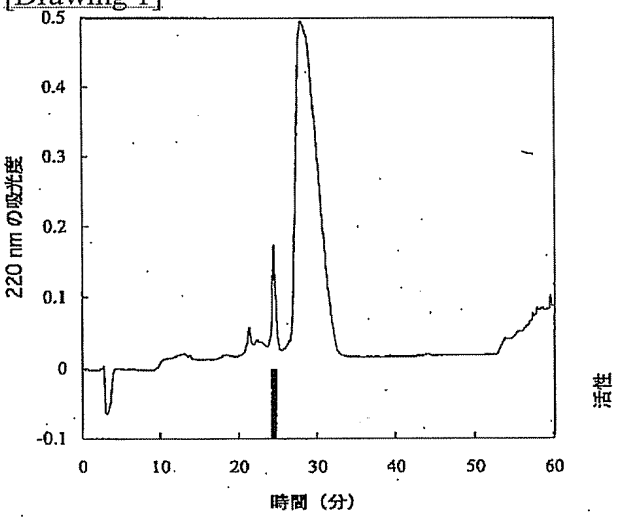
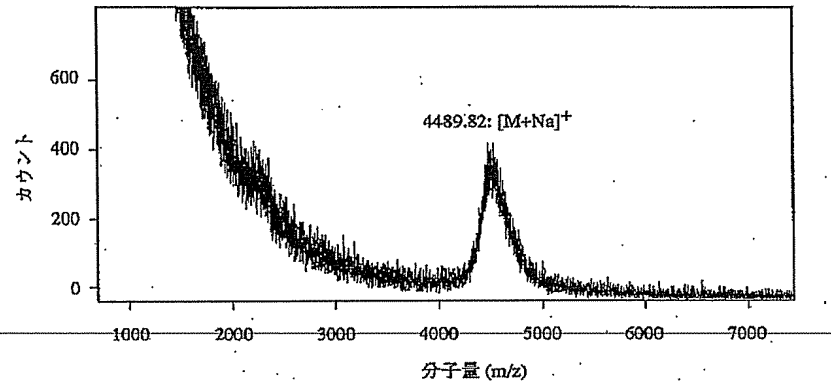
DRAWINGS

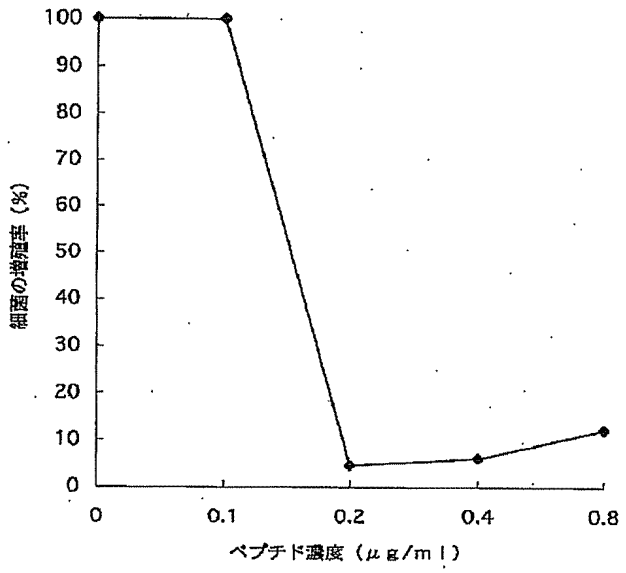
[Drawing 1]



[Drawing 2]



[Drawing 3]



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